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X-Linked Charcot-Marie-Tooth Disease: Molecular Analysis of Interfamilial Variability

Laura A. Niewiadomski and Thaddeus E. Kelly
Division of Medical Genetics, University of Virginia School of Medicine, Charlottesville

This report describes two families with type 1 Charcot-Marie-Tooth disease (CMTX), or hereditary motor sensory neuropathy type 1. Pedigree analysis is consistent with X-linked recessive inheritance in one family and X-linked dominant inheritance in the second. In the first family, a mutation in the connexin32 gene has been demonstrated and analyzed in family members. In the second family, linkage analysis is consistent with a mutation at the same locus. This report demonstrates the interfamilial variability in X-linked CMT and underscores the observation that regardless of the pattern of inheritance, X-linked CMT constitutes a single, variable disorder. © 1996 Wiley-Liss, Inc.

KEY WORDS: X-linked, Charcot-Marie-Tooth disease, connexin32, interfamilial variability

INTRODUCTION

A clear distinction between X-linked recessive and dominant traits often is not readily apparent. In hypophosphatemic rickets, heterozygous females demonstrate hyperphosphaturia and hypophosphatemia, but may or may not demonstrate short stature and radiographic evidence of rickets. This condition is classified as an X-linked dominant disorder. Most females heterozygous for hemophilia A will show lower than normal levels of factor VIII, but few have problems with spontaneous bleeding. This condition is classified as an X-linked recessive disorder. Thus, the designation, X-linked dominant, is used when most heterozygous females demonstrate phenotypic expression of their genotype and X-linked recessive when they do not.

In the tenth edition of *Mendelian Inheritance in Man*, McKusick [1992] lists X-linked dominant (302800) and X-linked recessive (302801) forms of Charcot-Marie-Tooth disease (CMT). Linkage studies have shown that both phenotypic forms of CMT map to the same locus at Xq13.1. [Gal et al., 1985; Corocs et al., 1992; Bergoffen et al., 1993a; Cochrane et al., 1994]. Subsequently, Bergoffen et al. [1993b] demonstrated mutations at the connexin32 locus in X-linked CMT. Additional mutations have been described by Fairweather et al. [1994] and Ionasescu et al. [1994].

This report describes two large families; in one family CMT segregates in an X-linked recessive fashion and in the other it segregates in an X-linked dominant fashion. In the first family a mutation at the connexin32 locus has been demonstrated and was used to complete the family study. In the second family, a mutation has not been found, but linkage data are presented which are consistent with a mutation at the same locus.

Pedigree Analysis

The pedigree for family 1 is shown in Figure 1; a classic X-linked recessive pattern of transmission is demonstrated.

The pedigree for family 2 is shown in Figure 2; a classic X-linked dominant pattern of transmission is demonstrated.

METHODS

Mutation Analysis

For the family exhibiting X-linked recessive inheritance, analysis of the connexin32 gene was facilitated by the creation of a restriction endonuclease site recognized by Dde I. The particular nonsense mutation results in truncation of the protein at amino acid 220, specifically Arg to a stop (CGA to TGA) codon [Bone et al., 1995]. A 666-bp fragment of the connexin32 coding region was amplified by the polymerase chain reaction (PCR) using the following primers and conditions: **Primer sequences**: (273–296) 5′ATCTCCCATGT GCGGCTGTGGTCC 3′ and (919–938) 5′TGCAGGTTG CCTGGTATGT 3′.

A 25-μl reaction volume using a Perkin Elmer kit contained 0.2 mM of dATP, dTTP, dCTP and dGTP, 10 nM of Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 15 pmol of each primer, and 1.0 unit of Taq DNA polymerase and 2 μCi of deoxycytidine 5′-[α 32 P]. One hundred fifty nanograms of genomic DNA were added, and 35 cycles of amplification carried out at 94°C for one minute, annealing at 63°C for one minute and extension at 72°C for one minute. Following amplification, the products

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Address reprint requests to Thaddeus E. Kelly, M.D., Ph.D., Box 386, University Hospital, Charlottesville, VA 22908.

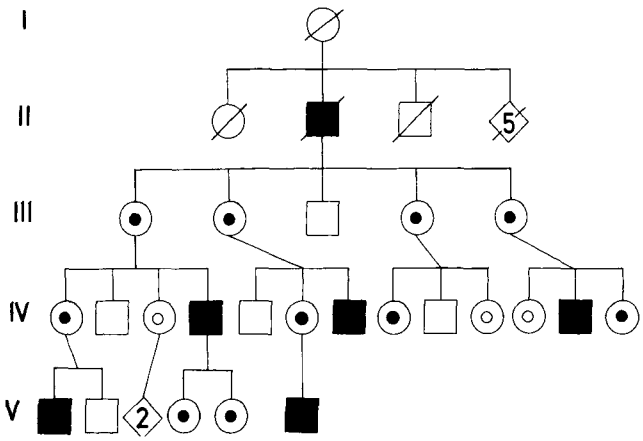

Fig. 1. Pedigree of family 1 showing a pattern of inheritance consistent with an X-linked recessive trait.

were incubated with Dde 1 at 37°C for three hours. The fragments were separated on 6% polyacrylamide sequencing gels. Following acetic acid-methanol fixation, the gels were placed in cassettes with Kodak X-omat film and developed after 4 hours exposure.

Linkage Analysis

After informed consent, DNA collected on family members in the X-linked dominant pedigree was analyzed initially for X-linked RFLPs by Southern blot and later for X-linked dinucleotide repeat polymorphisms by PCR much as described above. A total of 23 X-linked polymorphisms was studied in 46 family members over four generations. PCR primers were purchased from Research Genetics (Huntsville, AL) and reactions were carried out in 10-μl volumes much as described above. LOD scores were generated using MLINK of the LINKAGE package [Lathrop et al., 1984].

RESULTS

Mutation Analysis

The 666-bp PCR product from a normal X chromosome contains two Dde 1 restriction sites yielding

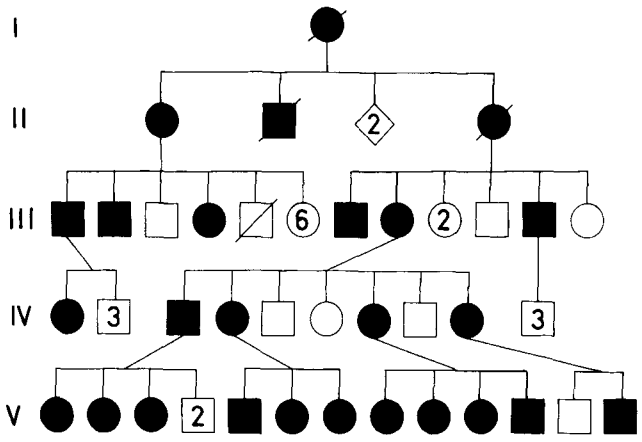

Fig. 2. Pedigree of family 2 showing a pattern of inheritance consistent with an X-linked dominant trait.

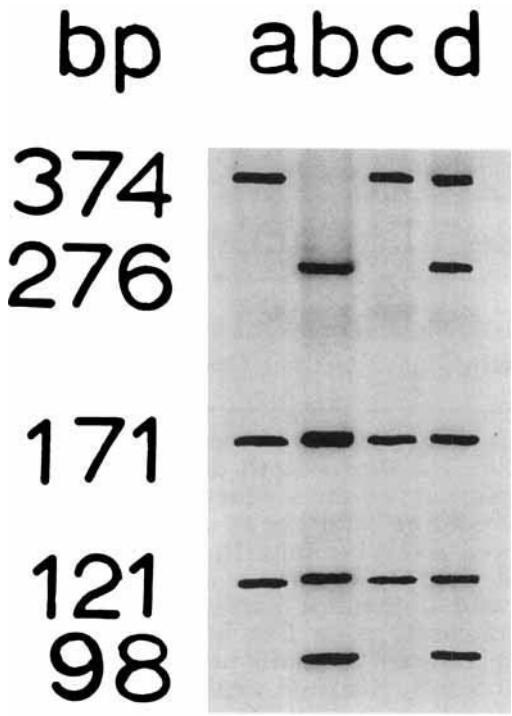

Fig. 3. Autoradiograph of the mutation in family 1. Unaffected males (**lane a**) and females (**lane c**) have a similar pattern with three DNA bands, while affected males (**lane b**) show four bands and heterozygous females show five bands (**lane d**).

bands of 374, 171 and 121 bp. X chromosomes bearing the mutation have a third Dde 1 site yielding four fragments: 276, 171, 121, and 98 bp. The patterns observed in affected and unaffected males and females are shown in Figure 3.

Mutation analysis was carried out on 23 members of family 1 (X-linked recessive pattern) and yielded the following results: five males were affected, five males were unaffected, ten females were heterozygotes and three females were homozygous normal. These results were consistent with the clinical diagnosis in each case.

Linkage Analysis

LOD scores generated by the data on family 2 are consistent with other reported families indicating linkage to the connexin32 locus at Xq13.1. The locus for the androgen receptor and the dinucleotide repeat polymorphism, DXS453, are most closely linked to the connexin32 locus [Bergoffen et al., 1993a; Cochrane et al., 1994]. The data are shown in Table I. Analysis of the

TABLE I. Lod Scores for X-Linked Polymorphisms in Family 2

Locus	Theta	Maximum lod score
DXS7	0.238	1.089
AR	0.000	5.118
DXS453	0.000	6.021
PGK1	0.118	2.443
DXS998	0.158	2.121
DXYS1	0.192	2.299
DXS3	0.303	1.143
DXYS2	0.222	0.639

TABLE II. Nerve Conduction Velocities

	Median motor NCV (m/sec) (normal = 57–71 m/sec)	Median sensory NCV (m/sec) (normal = 49–70 m/sec)
Affected males in family 1 (n = 5)	30–48	35–42
Affected females in family 1 (n = 10)	55–68	50–61
Affected males in family 2 (n = 8)	28–42	38–41
Affected females in family 2 (n = 12)	38–62	44–61

coding portions of the connexin32 gene in this family failed to demonstrate a mutation [Bone et al., 1995]. Analysis of noncoding portions of the connexin32 gene in this family continues.

Phenotype analysis. *Family 1.* The five affected males, ages 14 to 57 years, demonstrated clinical evidence of a peripheral neuropathy by their mid-teens. Thereafter, progression was slow and three over 45 years of age remained gainfully employed well into their forties with serious disability only apparent after age 50 years. None of the ten heterozygous females, ages 11 to 69 years, demonstrated clinical evidence of a peripheral neuropathy nor did they become disabled in later years. The males all demonstrated delayed nerve conduction velocities (NCV) in the median sensory and motor nerves much like that of males in family 2. However, the females in family 1 had low normal to normal NCV in the median sensory and motor nerves. Some females in family 2 showed moderate delays in NCV while other females had normal NCV. Results of NCV are shown in Table II.

Family 2. The phenotype in family 2 was the basis of a previous report by Phillips et al. [1985]. The eight affected males, ages 12 to 67 years, were clinically apparent by early teenage years with rapid progression thereafter. By their mid-twenties, these males were significantly disabled and few were gainfully employed past age 30 years. The twelve heterozygous females in this family, ages 16 to 83 years, were symptomatic before age 20 years with paresthesias and cold hands and feet being their predominant symptomatology. Muscle wasting was evident by 30 years of age and significant disability occurred by 45–50 years of age. NCVs are shown in Table II.

DISCUSSION

Charcot-Marie-Tooth disease (CMT) is a genetically heterogeneous disorder. Type 1 CMT, or hereditary motor sensory neuropathy I, is defined as a familial demyelinating, or hypertrophic, form of peripheral neuropathy characterized by peroneal muscular atrophy, absent deep tendon reflexes, delayed nerve conduction velocity and electromyographic evidence of denervation. Autosomal dominant forms of CMT secondary to mutations on chromosomes 1 and 17 account for the majority of cases of CMT. The prevalence of type 1 CMT is quoted at 12.9/100,000 [MacMillan and Harper, 1991] while the estimate for CMTX is 3.1/100,000 [Baraister, 1990]. Given the clinical variability of these three forms of CMT, it may be difficult to clinically distinguish X-linked dominant and autosomal dominant inheritance in small families. Nicholson and Nash [1993] analyzed 62 families with type 1 CMT. Forty-seven of these families demonstrated a duplication involving the peripheral myelin protein locus on chromosome 17p (CMT1A). Of the remaining 15 families, 8 were felt to represent probable CMTX, leaving 7 families presumably linked to chromosome 1 (CMT1B). They concluded that intermediate nerve conduction velocities serve as the best indicator of CMTX in small families. This observation was born out in family 2 as seen in Table II, but less so in family 1.

Among reported families with X-linked CMT, a pattern consistent with X-linked dominant transmission rather than X-linked recessive transmission predominates. These two families demonstrate that for X-linked traits, allelic mutations may be responsible for sufficient interfamilial variability so as to present a pedigree pattern consistent with X-linked dominant or X-linked recessive inheritance. Recognition of this fact has led to the tendency to refer to such disorders simply as being X-linked without reference to either a dominant or recessive pattern of transmission.

REFERENCES

Baraister M (1990): The genetics of neurological disorders. In Molunsky AG, Bobrow MB, Harper PS, Scriver C (eds): "Oxford Monograph on Medical Genetics." Vol. 18. 2nd ed. Oxford, U.K: Oxford University Press, pp 238–239.

Bergoffen J, Troffater J, Pericak-Vance MA, Haines JL, Chance PF, Fischbeck KH (1993a): Linkage localization of X-linked Charcot-Marie-Tooth disease. Am J Hum Genet 52:312–318.

Bergoffen J, Scherer SS, Wang S, Scott MO, Bone LJ, Paul Dl, Chen K, Lensch MW, Chance PF, Fischbeck KH (1993b): Connexin mutations in X-linked Charcot-Marie-Tooth disease. Science 262: 2039–2041.

Bone LJ, Dahl N, Lensch MW, Chance PF, Kelly TE, Le Guern E, Mage S, Parry G, Shapiro H, Wang S, Fischbeck KH (1995): New connexin32 mutations associated with X-linked Charcot-Marie-Tooth disease. Neurol 45:1863–1866.

Cochrane S, Bergoffen J, Fairweather ND, Muller E, Mostacciuolo ML, Monaco AP, Fischbeck KH, Haites NE (1994): X-linked Charcot-Marie-Tooth disease (CMTX1): A study of 15 families with 12 highly informative polymorphisms. J Med Genet 31:193–196.

Corocs IA, Lafreniere RG, Begy CR, Loch-Caruso R, Willard HF, Glover TW (1992): Refined localization of human connexin32 gene locus, GJB1, to Xq13.1. Genomics 13:479–480.

Fairweather N, Bell C, Cochrane S, Chelly J, Wang S, Mostacciuolo ML, Monaco AP, Haites NE (1994): Mutations in the connexin32 Gene

in X-linked Dominant Charcot-Marie-Tooth Disease (CMTX1). Hum Mol Genet 3:29–34.

Gal A, Mucke J, Theile H, Wieacker PF, Ropers HH, Wienker TF: X-linked dominant Charcot-Marie-Tooth disease (1985): Suggestion of linkage with a cloned DNA sequence from the proximal Xq. Hum Genet 70:38–42.

Ionasescu V, Searby C, Ionasescu R (1994): Point mutations of the connexin32 (GJB1) gene in X-linked dominant Charcot-Marie-Tooth neuropathy. Hum Mol Genet 3:355–358.

Lathrop ES, Lalouel JM, Julier C, Ott J (1984): Strategies for multilocus linkage analysis in humans. PNAS 81:3443–3446.

MacMillan JC, Harper PS (1991): Single gene neurological disorders in South Wales; an epidemiological study. Ann Neurol 30: 411–414.

McKusick VA (1992): "Mendelian Inheritance in Man." 10th ed. Baltimore: Johns Hopkins Press.

Nicholson G, Nash J (1993): Intermediate nerve conduction velocities serve to define X-linked Charcot-Marie-Tooth neuropathy families. Neurology 43:2558–2564.

Phillips LH, Kelly TE, Schnatterly P, Parker D (1985): Hereditary motor-sensory neuropathy (HMSN): Possible X-linked dominant inheritance. Neurology 35:498–501.